Enzyme-catalysed Formation of β-Amyrin from a Bicyclic Isomer of 2,3-Epoxysqualene

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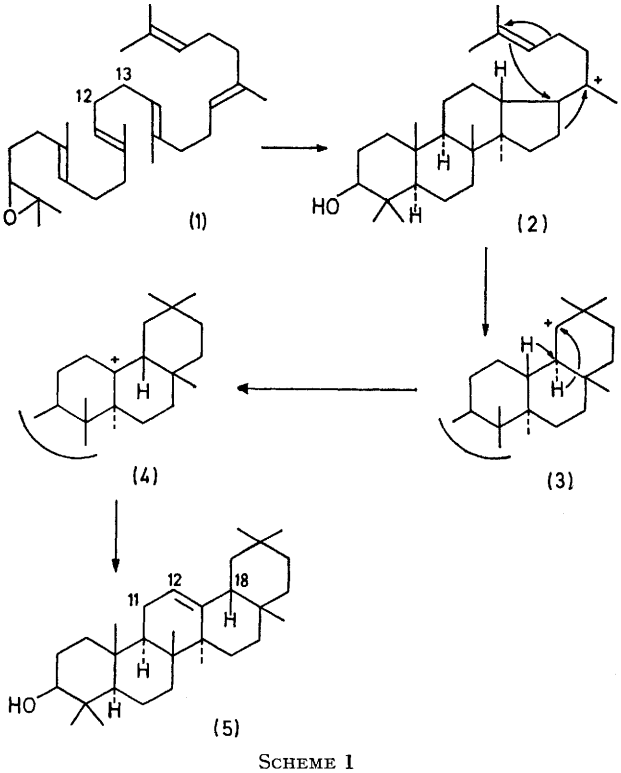
Summary A specifically tritiated specimen of the bicyclic epoxide (**11**) undergoes enzymic cyclization in a homogenate from *Pisum sativum* to give β-amyrin (**5**) without randomization of label.

THE biological formation of β-amyrin (**5**) in preparations of *P. sativum* is known to proceed through squalene[1] and the corresponding 2,3-epoxy-derivative (**1**).[2,3] A detailed mechanistic hypothesis, condensed in Scheme 1, had been offered[4] to account for this complex transformation; the postulated hydride shifts [*cf.* (**3**) → (**4**)] are well supported experimentally.[3,5]

Since the relevant cyclase is expected to accommodate *inter alia* the ionic entity (**4**), it could be argued that an alternative mode of generation of this ion on the enzyme surface, if at all possible, should also result in the formation of β-amyrin (**5**). Taking the extensive rearrangements required by the normal process in the area of the potential rings D and E [*cf.* (**2**) → (**3**)] as an indication of a pronounced flexibility of the corresponding enzyme tract,[6] it was felt that substrate (**11**) might prove well suited for a short-cut enzymic generation of the desired species (**4**).†

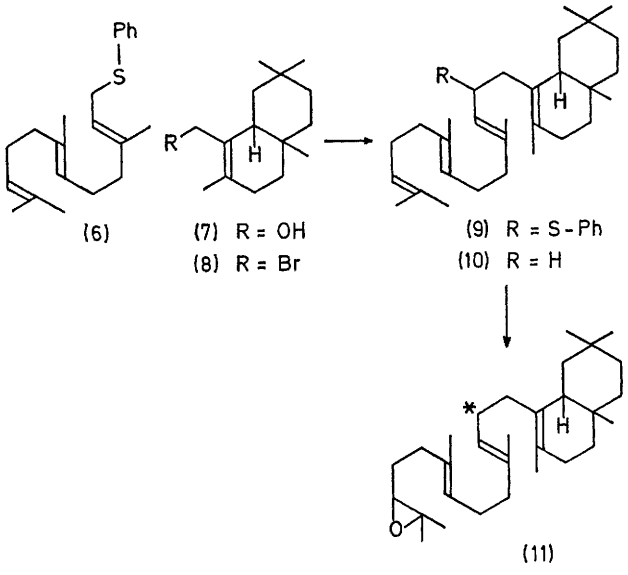
Treatment of the racemic bicyclic *cis*-alcohol (**7**)[8] with two equivalents of phosphorus tribromide in ether gave a quantitative yield of the bromide (**8**). The allylic anion generated from the *trans,trans*-farnesyl-thioether (**6**)[9] and butyl-lithium in THF–hexamethylphosphoramide (3 : 1) at −20° was alkylated[9] with (**8**) to give (**9**) (60%), and the latter converted[9] into (**10**) (80%) by treatment with lithium



SCHEME 1

† A similar situation has been previously exploited in the related case of lanosterol biosynthesis.[7]

(4 equiv.) in ethylamine at −25°. When (**10**) was submitted to the action of *N*-bromosuccinimide in aqueous THF, followed by potassium carbonate in methanol,[10] it was cleanly converted into (**11**), [δ (CCl_4) 1·23 and 1·26 (6H, s, epoxide-Me), 1·60 and 1·64 (9H, s, vinyl-Me), and 2·53 (1H,



SCHEME 2

t, *J* 6 Hz, epoxide-H)], essentially an equimolar mixture of the two possible diastereomeric racemates. Next, a sample of (**11**) specifically labelled with tritium at the starred position (spec. act. 2·33 × 10^{10} c.p.m./mmol) was prepared by the same method from labelled (**6**), itself available through quenching of the corresponding anion with tritiated water. As a standard for the biosynthetic experiments, 2,3-epoxy[12,13-^{3}H]squalene[9,10] (spec. act. 1·94 × 10^{10} c.p.m./mmol) was used.

The two labelled substrates (**1**) and (**11**) (3 mg each), were incubated in parallel experiments under nitrogen for 18 h at 28° with the crude homogenate from *P. sativum*.[1] After addition of 50 mg of cold carrier, the β-amyrin was purified by p.l.c., acetylated, and crystallized to constant activity, unchanged on conversion to the 3-keto[5] and the 12,13-epoxy[11] derivatives. The loss of activity observed on conversion into the 11-keto compound[12] (66·6% and 98% resp.) provides clear-cut evidence for the expected specific distribution of the label in both experiments. The percentage incorporations, corrected for the presence of the appropriate isomer in the starting material, were found to be 13% from (**1**) and 0·006% from (**11**). When δ-amyrin, the 13,18 double bond isomer of (**5**), was used as carrier in the experiment with (**11**) the recovered material was devoid of radioactivity. In contrast, van Tamelen's group has shown that chemical cyclization of (**11**), prepared in an independent synthesis, leads to an 8% yield of (±)-δ-amyrin, no β-amyrin being detected.[13]

In the light of the commonly accepted unifying scheme of triterpene biosynthesis[4] it seems highly unlikely that (**11**) represents a mandatory intermediate in the formation of β-amyrin. The observed transformation (**11**) → (**5**) should be taken rather as an illustration of the remarkable ability of the cyclizing enzyme to generate its normal product from an artificial substrate in a short-cut process which has been reduced to the formation of three rings.

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